

Spatial Distribution of Bacteria on Basalt Using SR-FTIR

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INTRODUCTION

Understanding the role of microorganisms on the fate and transport of contaminants is essential in the determination of risk assessment and the development of effective remediation strategies for contaminated sites. The microscale distribution of microorganisms on the surface of complex geologic media will affect the types and rates of biotransformations in contaminated environmental systems. In this study, we used synchrotron radiation-based Fourier transform infrared spectromicroscopy (SR-FTIR) at the Advanced Light Source (ALS) Beamline 1.4.3, Lawrence Berkeley National Laboratory (LBNL), to investigate the preferential attachment of *Burkholderia cepacia* G4 to the various mineral phases within basalt. SR-FTIR is a non-destructive, in situ analytical tool, that when coupled to an automated X, Y positioning stage, can provide surface mapping of biochemical functional groups.

MINERAL AND BASALT SPECIMENS

Basalt specimens were prepared from core samples. The center of the core was cut into 1" square blocks and then sliced into specimens 1 mm thick using a diamond saw blade and water as the lubricant. Mineral standards for the four major mineral phases in basalt (plagioclase, augite, ilmenite and olivine) were prepared in the same fashion. The specimens were sonicated and autoclaved prior to use. Basalt and mineral specimens were spectrally characterized using SR-FTIR prior to, and after, exposure to a bacterial growth culture for several days. SR-FTIR spectra

of the distinct mineral phases in the basalt were compared to spectra obtained for individual mineral standards.

BACTERIAL CELLS, CULTURES AND CONDITIONS

Burkholderia cepacia G4 was selected as the model organism for this study due to the fact that it is a common soil microorganism, and therefore representative of bacteria found in environmental systems. Microcosms consisting of basalt or mineral specimens and bacterial culture solution were placed on a rotary shaker (55 rpm) at 23 ° C for five days. The culture solutions were changed daily and new inoculum was added. At the end of the five days, the specimens were removed from solution and rinsed with phosphate buffer to remove any loose cells.

SR-FTIR SPECTROMICROSCOPY

SR-FTIR spectra were collected at Beamline 1.4.3 at the ALS, LBNL, Berkeley, CA. All SR-FTIR spectra were recorded in the 4000-650 cm^{-1} infrared region. This region contains absorbance features correlative to characteristic IR-active vibrational modes for common biomolecules such as nucleic acids, proteins and lipids, as well as identifying absorbance features for the basalt and mineral specimens. Spectral maps of the basalt surfaces were obtained by programming the microscope X-Y positioning stage to collect spectra at specific step locations and then extracting spatial information of functional groups based upon absorbance peak wavenumbers. Data was collected in single-beam reflectance mode with a spectral resolution of 4 cm^{-1} and 64 scans were co-added for Fourier transform processing for each spectrum. Each resulting spectrum was then ratioed to the spectrum of a gold slide to produce absorbance values. By extracting each individual spectrum within the mapping grid and comparing it to spectra collected on each of the four individual minerals, identification of the underlying mineralogy can be determined. Figure 1 shows the spatial distribution of bacteria on a basalt surface, based upon the occurrence of the protein amide I peak at $\sim 1650 \text{ cm}^{-1}$, and the preferential attachment by *B. cepacia* G4 to plagioclase. The total map area was 250 X 105 μm

with spectra collected every 25 μm along the X coordinate and every 15 μm along the Y coordinate. Correlative maps of the same surface area were constructed based upon the protein amide II peak at $\sim 1550\text{ cm}^{-1}$ and at least one other absorbance feature related to the presence of biomolecules, to insure the resulting map was due to the spatial distribution of bacteria and not an artifact of the mineralogy (data not shown).

RESULTS

Multiple SR-FTIR maps of basalt surfaces colonized by bacterial cultures showed preferential attachment by *B. cepacia* G4 to plagioclase within the basalt matrix. The mineral apatite ($\text{Ca}_5(\text{PO}_4)_3(\text{F}, \text{Cl}, \text{OH})$) is a common accessory mineral in igneous rocks and appears as inclusions in igneous plagioclase feldspars. Phosphorous is required by bacterial cells for the synthesis of nucleic acids and phospholipids. Scanning electron microprobe results indicate the presence of phosphorous in both the plagioclase within the basalt matrix and in the Ward's standard plagioclase specimen (data not shown). A recent study on feldspars as a source of nutrients determined that microorganisms extracted inorganic phosphorous from apatite inclusions in alkaline feldspars. It is highly likely that *B. cepacia* G4 preferentially attaches to the calcic plagioclase in order to access phosphorous from apatite inclusions.

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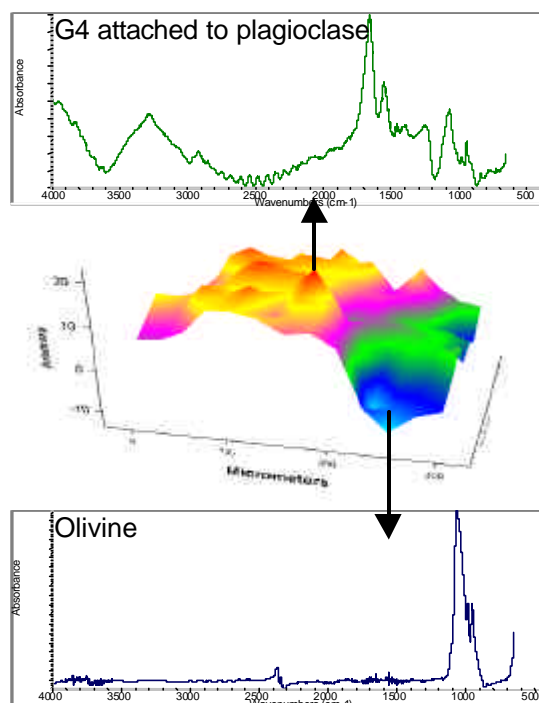


Figure 1.